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Licorice-induced pseudoaldosteronism in patients treated with Yokukansan preparations —identification of risk factors for hypokalemia—

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ABSTRACT

Objective: To evaluate serum potassium and rates of hypokalemia in patients treated with licorice-containing Japanese traditional Kampo medicines Yokukansan (YK) and Yokukansan-ka-chinpihange (YKCH).

Design: Retrospective case control study.

Setting: Patients receiving YK preparations for dementia and other psychiatric disorders in the University of Tsukuba Hospital in Japan.

Participants: Three hundred eighty-nine patients (male/female: 174/215, 68.6±16.1 years) who were treated with YK preparations for 231 days (range 6-2788 days). Patients whose potassium levels were less than <3.6 mEq/L before administration of YK preparations and drug noncompliant patients were excluded.

Main outcome measure: The occurrence rate of hypokalemia and assessment of the risk factor for YK-preparation induced hypokalemia.

Results: Out of 389 total patients, 94 (24.2%) developed hypokalemia (potassium levels <3.6 mEq/L) 34 days (range 1-1600 days) after administration of YK preparations. Thirty-six (38.3%) patients with hypokalemia had co-administration with lower potassium inducing drugs (LPIDs; diuretics, glucocorticoids, mineralocorticoids and glycyrrhizin), which was more frequent in the patients without hypokalemia (17.3%) (p<0.05). A logistic regression analysis identified the four risk factors for hypokalemia; co-administration of LPIDs (odds ratio 3.33, 95% confidence interval 1.89 to 5.85), YK administration (not YKCH) (2.99, 1.24 to 7.18), full dosed amounts (7.5g/day) (2.15, 1.26 to 3.65) and hypoalbuminemia at baseline (2.11, 1.22 to 3.66). Of the patients without LPIDs (n=302), 58 (19.2%) developed hypokalemia 42 days (range 1-1600 days) after administration of YK preparations. The risk factors for hypokalemia in this patient population were similar.

Conclusions: Serum potassium monitoring should be done at least monthly in patients with the risk factors of LPID co-administration, YK administration, and hypoalbuminemia.

Strengths and limitations of this study

- This is the first report to identify the risk factors for hypokalemia as an initial symptom of pseudoaldosteronism in patients treated with YK preparations containing small amounts of licorice (1.5g/day).
- The patients' data including their backgrounds and laboratory data can be investigated in the medical records precisely at the single clinical institution, University of Tsukuba Hospital.
- Since this is the retrospective case control study, blood sampling interval for assessing serum potassium and other laboratory data was not fixed.

INTRODUCTION

Yokukansan (YK) preparations YK and Yokukansan-ka-chimpihange (YKCH) are Japanese kampo (traditional) medicines consisting of 7 and 9 herbal extracts, respectively (Table 1), for the treatment of restlessness and agitation in children.[1] Current use of YK preparations focuses on the treatment of psychological symptoms of dementia (BPSD) in patients with Alzheimer's disease and Lewy body dementia.[1-10] This trend has altered the YK target patient population from children to the elderly in just the past decade.[11] An increase in adverse effects such as liver dysfunction, interstitial pneumonia, pseudoaldosteronism and rhabdomyolysis have been found in dementia patients, leading to the revision of the YK preparation package insert.[12] These adverse effects may be due to the change in target patient age (juvenile to elderly) and interactions with concomitant drugs being administered for the complications.[11,13]

Since both YK preparations contain licorice, a root of *Glycyrrhiza glabra*, they have licorice-induced pseudoaldosteronism characterized by hypertension and hypokalemia as their essential adverse effects.[14] This adverse effect has been ignored to this point because the licorice content of the preparation (1.5 g/day) is less than the 2.5 g/day which is considered to increase the risk of licorice-induced pseudoaldosteronism.[15] However, several observations revealed that the occurrence of hypokalemia caused by YK preparations is unexpectedly high and may develop into life-threatening events such as congestive heart failure and rhabdomyolysis, which required cessation of drug administration.[16-18]

In the present study, we retrospectively investigated the change in serum potassium levels in patients treated with YK preparations to assess the risk factor for hypokalemia as an initial symptom of pseudoaldosteronism.

METHODS

YK preparations

YK and YKCH compounds were obtained in a commercially available powdered form (Tsumura & Co., Tokyo, Japan) consisting of 7 and 9 herbal extracts, respectively (Table1). These traditional medicines are approved for medical use in Japan. Licorice content for both YK and YKCH were the same as the daily dose extracts of *Glycyrrhiza glabra*, (1.5 g) (Table1).

Patients and Study design

Three hundred eighty-nine patients (174 males and 215 females, 68.6±16.1 years) receiving YK preparations for BPSD of dementia or other psychiatric disorders were enrolled for the study at University of Tsukuba Hospital from March 2007 to January 2016 (Table 2). 184 patients were treated as outpatients and 205 were admitted to a hospital during YK administration. The patients received YK preparations for 6-2788 days (median 231 days). 323 patients were treated with YK and 66 with YKCH. YK preparations were given orally before or after meals at a full dose (2.5 g three times a day; 7.5 g/day) or a reduced dose (2.5 g once or twice a day; 2.5-5.0 g/day) based on patient symptoms. 229 patients (58.6%) received a full dose (7.5 g/day) of YK preparation. Noncompliant patients as well as those whose pre-administration serum potassium level was less than 3.6 mEq/L were excluded from the study. Any changes in laboratory data including serum potassium, sodium, chloride, aspartate aminotransferase, alanine aminotransferase, blood urea nitrogen, serum creatinine and albumin and co-medication were retrospectively investigated before and after administration of YK preparations.

Statistical analysis

Statistical parameters were ascertained by using SPSS software (International Business Machines Corp., Armonk, New York, USA). Statistical analyses were performed by Mann-Whitney test, chi-square test and logistic regression analysis. Logistic regression analysis was performed to identify risk factors for hypokalemia. A *p* value of less than 0.05 was considered to be statistically significant.

RESULTS

94 patients (24.2%) developed hypokalemia (potassium levels: <3.6 mEq/L) 34 (range 1-1600) days after administration of YK preparations (Table 2). Of the patients with hypokalemia, 36 (38.3%) were receiving concomitant doses of lower potassium inducing drugs (LPIDs; diuretics, glucocorticoids, mineralocorticoids and glycyrrhizin preparations), which was more frequent in the patients without hypokalemia (17.3%) (p<0.05) (Table 2). The patients with abnormal values in alanine aminotransferase, albumin and blood urea nitrogen at baseline were significantly higher for the hypokalemia group than the non-hypokalemia group (18.1 vs. 10.2%, 50.0 vs. 29.2% and 39.4 vs. 26.4%, p<0.05), respectively. A higher rate of hypoalbuminemia (albumin levels: <3.8 g/L) was also observed in patients with hypokalemia (45.7% vs. 28.8%, p<0.05) at minimum potassium levels during the administration of YK preparations (data not shown).

Logistic regression analysis identified the four risk factors for hypokalemia: co-administration of LPIDs (odds ratio 3.33, 95% confidence interval 1.89 to 5.85), YK administration (odds ratio 2.99, 95% CI 1.24 to 7.18), hypoalbuminemia (odds ratio 2.15, 95% CI 1.26 to 3.65) and full dose administration of YK preparations (7.5 g/day) (odds ratio 2.11, 95% CI 1.22 to 3.66) (Figure 1-a). To assess the effects of LPID co-administration on occurrence of hypokalemia, the dosing period of YK preparations until development of hypokalemia was compared between groups with and without LPIDs (Figure 2). YK patients

treated with concomitant LPIDs showed a shorter time-to-occurrence for hypokalemia than those without concomitant LIPDs (Figure 2).

To eliminate the effects of LPID co-administration on the occurrence of hypokalemia, we further analyzed the data in 302 patients treated without LPIDs (Table 3). Out of this number of patients, 58 (19.2%) developed hypokalemia 42 days (range 1-1600 days) after administration of YK preparations (Table 3). Significant differences in age and body weight were observed between patients with hypokalemia and non-hypokalemia (72.8±12.8 vs. 68.3±15.1 years, 49.9±13.2 vs. 55.0±13.8 kg, $p<0.05$) (Table 3). Higher rates of YK administration (not YKCH) and full-dosed amounts were also observed in the patients with hypokalemia (Table 3). The rate of hypoalbuminemia in patients with hypokalemia was significantly higher than that for non-hypokalemia at both baseline (44.8 vs. 27.5%, $p<0.05$) and when potassium level was minimal (46.6 vs. 26.6%, $p<0.05$) (data not shown). The logistic regression analysis identified four risk factors for hypokalemia: YK administration (4.77, 1.09 to 20.80), full-dosed amounts (2.65, 1.30 to 5.40), hypoalbuminemia (2.13, 1.11 to 4.07) and female (1.94, 1.02 to 3.69) (Figure 1-b).

Nine patients discontinued YK preparations due to hypokalemia and each possessed the risk factors indicated in Figure 1 (Table 4). Eight patients (except for Case 7) had multiple risk factors. Cases 1 and 2 who developed severe hypokalemia with potassium level < 2.1 mEq/L had been co-administered thiazide diuretic and presented with rhabdomyolysis, respectively (Table 4).

DISCUSSION

Both YK preparations contain 1.5 g/day of licorice extracts (Table 1), which is much less than Shakuyakukanzo-to (6.0 g/day of licorice) possessing the highest risk for pseudoaldosteronism among the Kampo-medicines.[19-21] However, the Japanese Adverse

Drug Event Report (JADER), a spontaneous adverse events reporting system, currently reports that YK-induced pseudoaldosteronism rates are comparable with those of Shakuyakukanzo-to, even though the possible risk should be low in terms of the licorice contents.[19,20] Present results seemed to confirm the JADER's reports; hypokalemia was found in high frequency, with 24.2% of the patients having been treated by YK preparations. This rate is comparable with a previous investigation in elderly patients, where 17% of patients treated with YK developed hypokalemia.[22]

On the other hand, an adverse drug reactions (ADRs) frequency investigation on YK for ethical use reported that hypokalemia occurred in 1.3% patients treated with YK,[13] which was considerably lower than our observation. Several factors may explain this difference in the occurrence rate of hypokalemia. One possible reason is patient background in terms of disease severity, complications and concomitant drug administration. 80% of the subjects in the ADRs investigation were outpatients and 61.9% of the patients had no complications and no medication for dementia.[13] On the other hand, this study enrolled patients who presented with complicating psychiatric disorders (48.8%) and received various medications, including LPIDs such as diuretics, glucocorticoids, mineralocorticoids and glycyrrhizin preparations. Another possible reason is observation periods between the studies. The ADRs frequency investigation did not track adverse events longer than 52 weeks after starting YK administration.[13]

Licorice induced-pseudoaldosteronism due to Kampo-medicines can escalate into a serious event that makes hospitalization necessary. The mechanism seems to be clear.[23-26] Glycyrrhetinic acid (GA), a metabolite of glycyrrhizin (GL) contained in licorice, has been found to be the major substance for pseudoaldosteronism. GA inhibits 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD 2), which catalyzes the conversion of cortisol to cortisone and prevents the binding of cortisol to the mineralocorticoid receptor (MCR) in the

mineralocorticoid target tissues. This inhibition leads to increased cortisol levels in the tissues and excess the cortisol binding to the MCR with same affinity of aldosterone.[23,24] The MCR activation increases sodium reuptake and inhibits potassium reabsorption in the kidney, resulting in hypertension, metabolic alkalosis and hypokalemia.[25] Monitoring of serum potassium levels, therefore, is useful for early detection and assessing the severity of pseudoaldosteronism. Our present results suggested that serum potassium levels should be checked at the first month after starting YK preparations, because the median for hypokalemia onset was 34 days after administration in patients with and without LPID (Figure 2).

We found four risk factors associated with hypokalemia in patients with heterogeneous clinical backgrounds (Figure 1). Patients co-administered with LPIDs were 3.3 times more likely to develop to hypokalemia than with YK preparation alone (Figure 1-a). LPID co-administration speeds up the hypokalemia onset time by 15 days compared with YK preparation alone (Figure 2). Among the LPIDs, loop and thiazide diuretics should be carefully noted because they are frequently prescribed for dementia patients with hypertension (data not shown). Severe hypokalemia cases with low potassium levels of 1.9 mEq/L had received thiazide diuretics concomitantly (Table 4).

The risk of hypokalemia during YK treatment was 2.99 times higher than that of YKCH. One conceivable explanation of this finding may be due to the difference in GL contents between YK and YKCH even though the licorice contents are the same (1.5 g/day) as shown in Table 1. Kampo-medicines, including YK preparations, were lyophilization products consisting of herbal extracts as showed in Table 1. The mixture of 7 or 9 plants are added to water and boiled, filtered, concentrated and then the resulting decoctions are further lyophilized to yield the dry extract for making YK preparations. In this manufacturing process, the wet extraction rate of GL may be different between YK and YKCH due to difference in the combination of plants. Higher GL content for YK might therefore present a higher risk of

hypokalemia compared with YKCH.

Patients with hypoalbuminemia had a 2.15 times higher rate of hypokalemia (Figure 1-a). Since 99.9% of circulating GA are bound to albumin,[27] hypoalbuminemia may increase the unbound fraction of GA through pharmacokinetic alteration, resulting in an enhancement of the pharmacological actions of GA. These results are the first report that hypoalbuminemia is a possible risk factor for licorice-induced hypokalemia.

The occurrence of hypokalemia might be dose dependent in patients treated with YK preparations, because full-dose YK preparations (7.5 g/day) increased the risk more than 2 times compared with a reduced dose (Figure 1-a, b). This observation is consistent with previous reports suggesting that licorice-induced pseudoaldosteronism was found in a dose-dependent manner.[20,21] Since a majority of the dementia patients taking YK preparations are elderly, the reduced dose is recommended for any patients carrying the risk factors of pseudoaldosteronism. Although age was not identified as a risk factor for hypokalemia in the present study, this might be due a lack of comparison, as most of the patients investigated were elderly (68.6±16.1 years old). Initiation of full-dose YK preparations would therefore be avoided in elderly patients whose 11β-HSD activity might be low due to age-dependent decline in kidney function.[28] In fact, 7 of 9 patients who discontinued YK preparations due to hypokalemia were over 70 years old and carried multiple risk factors of hypokalemia (Table 4).

CONCLUSION

Hypokalemia was found at an unexpectedly high rate in patients under treatment with YK preparations even though the licorice content is relatively small. Four risk factors were found to be important in elderly patients under long term treatment with YK preparations: LPIDs co-administration, YK administration, hypoalbuminemia and full dosage

administration (7.5 g/day). It is recommended that serum potassium monitoring should be done at least monthly for safe use of YK preparations in patients with multiple risk factors.

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Contributors

SS, TA, AT and MH designed and supervised the study. TA and AT selected the patients for this study. SS and MH corrected the data and carried out statistical analyses. SS and MH drafted the original manuscript and all authors checked and revised the manuscript. SS and MH are the guarantors.

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Competing interests

We declare no competing interests.

Ethical approval

This study was approved by The Ethical Committee of the University of Tsukuba Hospital.

Data sharing

The full dataset is available from the corresponding author.

Transparency

The lead author affirms that the manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned have been explained.

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Table 1. Components of YK preparations

Constituent herbs	Weight (g/day)	
	YK	YKCH
Atractylodes lancea rhizome	4.0	4.0
Poria sclerotium	4.0	4.0
Japanese Angelica radix	3.0	3.0
Cnidium rhizome	3.0	3.0
Uncaria hook	3.0	3.0
Bupleurum root	2.0	2.0
Glycyrrhiza radix	1.5	1.5
Pinelliae tuber	-	5.0
Aurantii nobilis pericarpium	-	3.0

Table 2. Demographic data of the subjects

	Hypokalemia	Non-hypokalemia
Number of patients (male / female)	94 (35/59)	295 (139/156)
Age (years)	69.5±16.7	68.2±15.9
Body weight (kg) [a]	51.2±12.8	54.6±14.5
Disease (dementia / other psychiatric disorder)	42/52	157/138
YK preparations treatment		
YK / YKCH	86/8 *	237/58
Full dose	66 (70.2%) *	163 (55.3%)
Dosing period (days)	169 (8-2280) *	266 (6-2788)
Dosing period until hypokalemia (days)	34 (1-1600)	-
Co-administration of LPIDs	36 (38.3%) *	51 (17.3%)
Diuretics (loop / thiazide)	10/4	15/7
Glucocrticoids / Mineralcorticoid	18/0	23/2
Glycyrrhizin preparation	7	18
Serum potassium (mEq/L)		
Baseline	4.0±0.3	4.2±0.4
Minimum	3.2±0.3 *	4.1±0.3
Laboratory abnormality at baseline [b]		
Aspartate aminotransferase (U/L)	11 (11.7%)	24 (8.1%)
Alanine aminotransferase (U/L)	17 (18.1%) *	30 (10.2%)
Albumin (g/dL)	47 (50.0%) *	86 (29.2%)
Blood urea nitrogen (mg/dL)	37 (39.4%) *	78 (26.4%)
Creatinine (mg/dL)	28 (29.8%)	107 (36.3%)
Sodium (mEq/L)	7 (7.4%)	17 (5.8%)
Chloride (mEq/L)	11 (11.7%)	39 (13.2%)

The data are presented as number of patients, mean±S.D. or median (range).
Significant differences were observed : * $p<0.05$ versus non-hypokalemia.
[a] The number of patients whose weight is present is 85 with hypokalemia and 224 with non-hypokalemia.
[b] The number of patients with abnormal laboratory data at baseline.
The normal range for laboratory data are as follows;
Aspartate aminotransferase: 8.0-38.0 U/L
Alanine aminotransferase: 4.0-44.0 U/L
Albumin: 3.8-5.3 g/dL
Blood urea nitrogen: 8.0-20.0 mg/dL
Creatinine: 0.61-1.04 mg/dL in male, 0.47-0.79 mg/dL in female
Sodium: 135.0-147.0 mEq/L
Chloride: 98.0-108.0 mEq/L

Table 3. Comparison of baseline characteristics and laboratory data in patients treated without LPIDs.

	Hypokalemia	Non-hypokalemia
Number of patients (male / female)	58 (21/37)	244 (118/126)
Age (years)	72.8±12.8 *	68.3±15.1
Body weight (kg) [a]	49.9±13.2 *	55.0±13.8
Disease (dementia / other psychiatric disorder)	33/25	135/109
YK preparations treatment		
YK / YKCH	55/3 *	200/44
Full dose	43 (74.1%) *	135 (55.3%)
Dosing period (days)	181 (8-2280)	294 (6-2788)
Dosing period until hypokalemia (days)	42 (1-1600)	-
Serum potassium (mEq/L)		
Baseline	4.1±0.4	4.2±0.4
Minimum	3.2±0.3 *	4.1±0.3
Laboratory abnormality at baseline [b]		
Aspartate aminotransferase (U/L)	10 (17.2%) *	16 (6.6%)
Alanine aminotransferase (U/L)	12 (20.7%) *	22 (9.0%)
Albumin (g/dL)	26 (44.8%) *	67 (27.5%)
Blood urea nitrogen (mg/dL)	21 (36.2%)	59 (24.2%)
Creatinine (mg/dL)	15 (25.9%)	86 (35.2%)
Sodium (mEq/L)	4 (6.9%)	11 (4.5%)
Chloride (mEq/L)	4 (6.9%)	29 (11.9%)

The data are presented as number of patients, mean±S.D. or median (range).
Significant differences were observed : * $p<0.05$ versus non-hypokalemia.
[a] The number of patients whose weight is present is 47 with hypokalemia and 174 with non-hypokalemia.
[b] The number of patients with abnormal laboratory data at baseline.
The normal range for laboratory data are as follows;
Aspartate aminotransferase: 8.0-38.0 U/L
Alanine aminotransferase: 4.0-44.0 U/L
Albumin: 3.8-5.3 g/dL
Blood urea nitrogen: 8.0-20.0 mg/dL
Creatinine: 0.61-1.04 mg/dL in male, 0.47-0.79 mg/dL in female
Sodium: 135.0-147.0 mEq/L
Chloride: 98.0-108.0 mEq/L

Table 4. Characteristics of nine patients who were discontinued from YK preparations for hypokalemia

Case	YK preparations	YK preparations dose (g/day)	Dosing period until hypokalemia (days)	Minimum value of serum potassium (mEq/L) (reduction)	Baseline albumin (g/dL)	Co-medication and symptoms	Number of risk factors
1	YK *	7.5 *	205	1.9 (-2.5)	4.1	Hydrochlorothiazide * Edema	3
2	YK *	7.5 *	554	2.0 (-3.0)	4.1	Rhabdomyolysis	3
3	YK *	7.5 *	24	2.8 (-2.0)	2.5 *	-	4
4	YK *	5.0	160	2.8 (-1.4)	-	-	2
5	YK *	7.5 *	8	2.9 (-1.7)	2.1 *	Rikkunshito [a] *	4
6	YKCH	7.5 *	161	2.9 (-1.1)	3.7 *	-	3
7	YK *	5.0	237	3.3 (-0.6)	-	Edema Hypertension	1
8	YK *	5.0	26	3.3 (-0.5)	2.6 *	-	3
9	YKCH	7.5 *	26	3.5 (-2.5)	3.4 *	-	3

* Risk factors for hypokalemia are as follows;
patient with LPIDs: LPIDs, YK, hypoalbuminemia, full dose
patient without LPIDs: female, YK, hypoalbuminemia, full dose

[a] Other Kampo-medicine including licorice.

FIGURE LEGENDS

Figure 1. Adjusted odds ratio for hypokalemia in patients treated with YK preparations.

a: patients co-administered with LPIDs, b: patients co-administered without LPID co-administration

Figure 2. Difference in the occurrence rate of hypokalemia between patients with and without LPID co-administration.

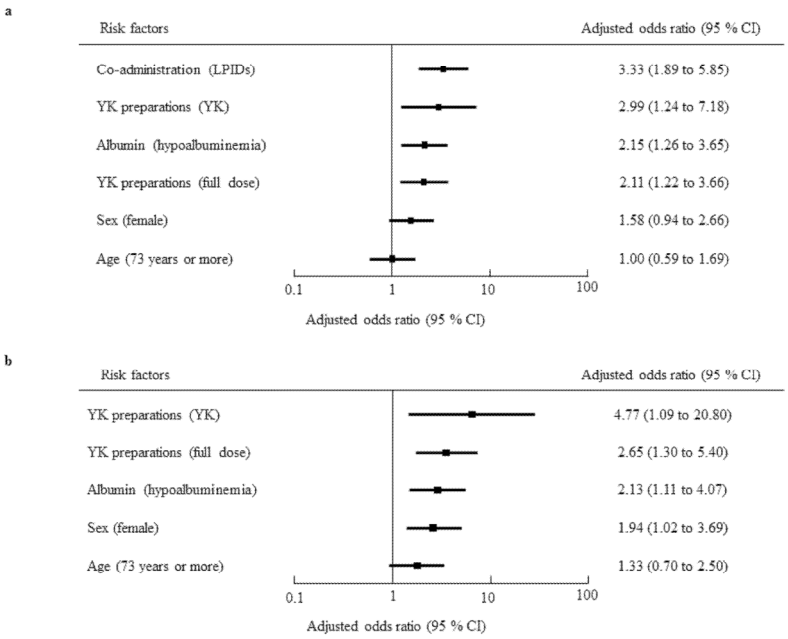

Figure 1. Adjusted odds ratio for hypokalemia in patients treated with YK preparations.
a: patients co-administered with LPIDs, b: patients co-administered without LPID co-administration

420x297mm (300 x 300 DPI)

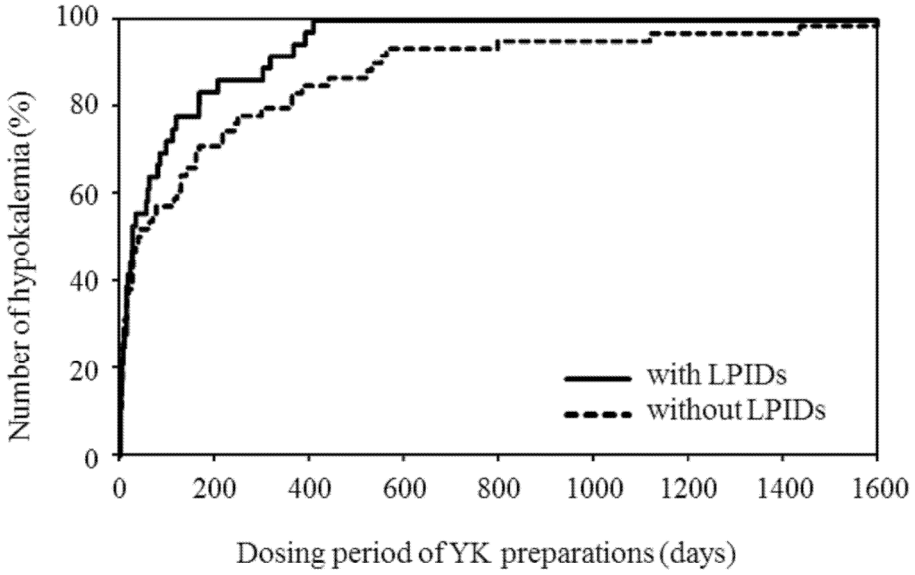

Figure 2. Difference in the occurrence rate of hypokalemia between patients with and without LPID co-administration.

420x297mm (300 x 300 DPI)

STROBE Statement—Checklist of items that should be included in reports of ***case-control studies***

	Item No	Recommendation	Reported on page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	Page 2, line 5
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	Page 2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	Page 4, line 4-20
Objectives	3	State specific objectives, including any prespecified hypotheses	Page 4, line 13-23
Methods			
Study design	4	Present key elements of study design early in the paper	Page 5, line 9-23
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	Page 5, line 10-23
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls	Page 5, line 10-20
		(*b*) For matched studies, give matching criteria and the number of controls per case	Not applicable
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	Page 5, line 13-18, 20-23
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	Page 5, line 20-23
Bias	9	Describe any efforts to address potential sources of bias	Not applicable
Study size	10	Explain how the study size was arrived at	Not applicable
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	Not applicable
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	Page 6, line 1-5
		(*b*) Describe any methods used to examine subgroups and interactions	Not applicable
		(*c*) Explain how missing data were addressed	Not applicable
		(*d*) If applicable, explain how matching of cases and controls was addressed	Not applicable
		(*e*) Describe any sensitivity analyses	Not applicable

Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	Page 6, line 8-9, page 7, line 3-4, table 2 and 3
		(b) Give reasons for non-participation at each stage	Not applicable
		(c) Consider use of a flow diagram	Not applicable
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Page 6, line 8-18, page 7, line 3-10, table 2 and 3
		(b) Indicate number of participants with missing data for each variable of interest	Table 2 and 3
Outcome data	15*	Report numbers in each exposure category, or summary measures of exposure	Table 2 and 3
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Figure 1
		(*b*) Report category boundaries when continuous variables were categorized	Not applicable
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	Not applicable
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	Not applicable
Discussion			
Key results	18	Summarise key results with reference to study objectives	Page 8, line 4-5, page 9, line 9-10
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	Not applicable
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	Page 7, line 23-25, page 8, line 7
Generalisability	21	Discuss the generalisability (external validity) of the study results	Not applicable
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	Page 11, line 13

*Give information separately for cases and controls.

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Licorice-induced hypokalemia in patients treated with Yokukansan preparations—identification of the risk factors in a retrospective cohort study—

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Licorice-induced hypokalemia in patients treated with Yokukansan preparations—identification of the risk factors in a retrospective cohort study—

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Key words: hypokalemia, pseudoaldosteronism, yokukansan preparations, licorice containing kampo-medicine, risk factors

ABSTRACT

Objective: To evaluate serum potassium and rates of hypokalemia in patients treated with licorice-containing Japanese traditional Kampo medicines Yokukansan (YK) and Yokukansan-ka-chinpihange (YKCH).

Design: Retrospective cohort study.

Setting: Patients receiving YK preparations for dementia and other psychiatric disorders in the University of Tsukuba Hospital in Japan.

Participants: Three hundred eighty nine patients (male/female: 174/215, 68.6±16.1 years) who were treated with YK preparations for 231 days (range 6 to 2788 days). Patients whose potassium levels were less than 3.6 mEq/L before administration of YK preparations and drug noncompliant patients were excluded.

Main outcome measure: The occurrence rate of hypokalemia and assessment of the risk factor for YK-preparation induced hypokalemia.

Results: Out of 389 total patients, 94 (24.2%) developed hypokalemia (potassium levels <3.6 mEq/L) 34 days (range 1 to 1600 days) after administration of YK preparations. Thirty six (38.3%) patients with hypokalemia had co-administration with lower potassium inducing drugs (LPIDs; diuretics, glucocorticoids, mineralcorticoids and glycyrrhizin), which was more frequent in the patients without hypokalemia (17.3%) (p<0.05). Cox proportional hazard model identified the four risk factors for hypokalemia; YK administration (not YKCH) (hazard ratio 3.093, 95% confidence interval 1.408 to 6.798), co-administration of LPIDs (2.743, 1.754 to 4.289), hypoalbuminemia at baseline (2.145, 1.360 to 3.384) and full dosed amounts (7.5g/day) (1.600, 1.005 to 2.549).

Conclusions: Serum potassium monitoring should be done at least monthly in patients with the risk factors of LPIDs co-administration, YK administration, hypoalbuminemia and full dosage administration.

Strengths and limitations of this study

- This is the first report to identify the risk factors for hypokalemia as an initial symptom of pseudoaldosteronism in patients treated with YK preparations containing small amounts of Glycyrrhiza (1.5g/day).
- The patients' data including their backgrounds and laboratory data can be investigated in the medical records precisely at the single clinical institution, University of Tsukuba Hospital.
- Since this is the retrospective cohort study, blood sampling interval for assssing serum potassium and other laboratory data was not fixed.

INTRODUCTION

Yokukansan (YK) preparations YK and Yokukansan-ka-chimpihange (YKCH) are Japanese kampo (traditional) medicines consisting of 7 and 9 crude drug extracts, respectively (Table 1), for the treatment of restlessness and agitation in children.[1] Current use of YK preparations focuses on the treatment of psychological symptoms of dementia (BPSD) in patients with Alzheimer's disease and Lewy body dementia.[1-10] This trend has altered the YK target patient population from children to the elderly in just the past decade.[11] An increase in adverse effects such as liver dysfunction, interstitial pneumonia, pseudoaldosteronism and rhabdomyolysis have been found in dementia patients, leading to the revision of the YK preparation package insert.[12] These adverse effects may be due to the change in target patient age (juvenile to elderly) and interactions with concomitant drugs being administered for the complications.[11,13]

Since both YK preparations contain licorice as the crude drug Glycyrrhiza, they have licorice-induced pseudoaldosteronism characterized by hypertension and hypokalemia as their essential adverse effects.[14] This adverse effect has been ignored to this point because the Glycyrrhiza content of the preparation (1.5 g/day) is less than the 2.5 g/day which is considered to increase the risk of licorice-induced pseudoaldosteronism among the Kampo-medicines containing Glycyrrhiza.[15] (Table 2) However, several observations revealed that the occurrence of hypokalemia caused by YK preparations is unexpectedly high and may develop into life-threatening events such as congestive heart failure and rhabdomyolysis, which required cessation of drug administration.[16-18]

In the present study, we retrospectively investigated the change in serum potassium levels in patients treated with YK preparations to assess the risk factor for hypokalemia as an initial symptom of pseudoaldosteronism.

METHODS

YK preparations

YK preparations (YK and YKCH) were obtained in a commercially available granule (Tsumura & Co., Tokyo, Japan) consisting of the extract prepared from the mixture of 7 and 9 crude drugs, respectively (Table1). These traditional medicines are approved for medical use in Japan. The daily dose of both YK preparations is 7.5 g/day as the granule, which contains a dried extract of the mixed crude drugs of YK (3.25 g) or YKCH (4.5 g) as shown in Table 1. Glycyrrhiza content for both YK and YKCH were the same as the daily dose extracts (1.5 g) (Table1, 2).

Patients and Study design

Three hundred eighty-nine patients (174 males and 215 females, 68.6±16.1 years) receiving YK preparations for BPSD of dementia or other psychiatric disorders were enrolled for the study at University of Tsukuba Hospital from March 2007 to January 2016 (Table 3). 184 patients were treated as outpatients and 205 were admitted to a hospital during YK administration. 323 patients were treated with YK and 66 with YKCH for 231 days (range 6 to 2788 days). YK preparations were given orally before or after meals at a full dose (2.5 g three times a day; 7.5 g/day) or a reduced dose (2.5 g once or twice a day; 2.5-5.0 g/day) based on patient symptoms. 229 patients (58.9%) received a full dose of YK preparation. Noncompliant patients as well as those whose pre-administration serum potassium level was less than 3.6 mEq/L were excluded from the study. Changes in laboratory data including serum potassium, sodium, chloride, aspartate aminotransferase, alanine aminotransferase, blood urea nitrogen, serum creatinine and albumin and co-medication were retrospectively investigated before and after administration of YK preparations in the medical records.

Statistical analysis

Statistical parameters were ascertained by using SPSS software (International Business Machines Corp., Armonk, New York, USA). Statistical analyses were performed by Mann-Whitney test, and chi-square test for comparing the difference between hypokalemia and non-hypokalemia group. Cut-off value for discriminating between haypokalemia and non-hypokalemia was determined by Receiver Operatorating Characteristic curve. Intergroup differences, patients treated with and without LPIDs, were analyzed by the log-rank test in Kaplan-Meier method. Cox proportional hazard model was employed to identify risk factors for hypokalemia. A p value of less than 0.05 was considered to be statistically significant.

RESULTS

94 patients (24.2%) developed hypokalemia (potassium levels: <3.6 mEq/L) during observational period (Table 3). The median days for developing hypokalemia was 34 (range 1 to 1600 days) after administration of YK preparations (Table 3), at which the cumulative rate of hypokalemia was 12.7% in Kaplan-Meier analysis (Figure 1).

Significant difference in administration of type of YK preparations (YK or YKCH), dosed amount and the dosing period was observed between hypokalemia and non-hypokalemia. Hypokalemia group received higher rate of YK (not YKCH) and full dosed amounts (compared with non-hypokalemia (91.5 vs. 80.3% and 70.2 vs. 55.3%, respectively, p<0.05) (Table 3). The dosing period in hypokalemia was significantly shorter than non-hypokalemia (169, range 8 to 2280 vs. 266, range 6 to 2788 days, p<0.05) (Table 3).

Of the patients with hypokalemia, 36 (38.3%) were receiving concomitant doses of lower potassium inducing drugs (LPIDs; diuretics, glucocorticoids, mineralocorticoids and glycyrrhizin preparations), which was more frequent in the patients without hypokalemia (17.3%) (p<0.05) (Table 3). Mean serum potassium at baseline in hypokalemia group was

also lower than non-hypokalemia (p<0.001), even though their values were in normal range. The reduction for serum potassium (delta potassium) after administration YK preparations was remarkable in hypokalemia compared with non-hypokalemia group (-0.7: -3.0 to -0.1 vs. -0.1: -1.3 to 1.1, p<0.001) (Table 3).

The patients with abnormal values in alanine aminotransferase, albumin and blood urea nitrogen at baseline were significantly higher for the hypokalemia group than the non-hypokalemia group (14.9 vs. 9.8%, 50.0 vs. 29.2% and 39.4 vs. 26.4%, p<0.05), respectively. A higher rate of hypoalbuminemia (albumin levels: <3.8 g/L) was also observed in patients with hypokalemia (45.7 vs. 28.8%, p<0.05) at minimum potassium levels during the administration of YK preparations (data not shown).

Cox proportional hazard model based on univariable and multivariable analysis identified four risk factors for YK-preparations induced hypokalemia: YK administration (not YKCH) (hazard ratio 3.093, 95% confidence interval 1.408 to 6.798), co-administration of LPIDs (HR 2.743, 95% CI 1.754 to 4.289), hypoalbuminemia at baseline (HR 2.145, 95% CI 1.360 to 3.384) and full dose administration of YK preparations (7.5 g/day) (HR 1.600, 95% CI 1.005 to 2.549) (Table 4). On the other hand, baseline potassium levels of ≥4.1 mEq/L, which were obtained optimal cut-off value to predict non-hypokalemia, were the reverse factor for hypokalemia (HR 0.450, 95% CI 0.288 to 0.702) (Table 4).

To assess the effects of LPIDs co-administration on occurrence of hypokalemia, the observational period of YK preparations until development of hypokalemia was compared between groups with and without LPIDs (Figure 2). Patients treated with concomitant LPIDs showed a shorter time-to-occurence for hypokalemia than those without concomitant LIPDs (Figure 2) (p<0.001).

Nine patients discontinued YK preparations due to hypokalemia and each possessed the risk factors indicated in Table 5. Seven patients (except for Case 4 and 7) had multiple risk

factors. Cases 1 and 2 who developed severe hypokalemia with potassium level < 2.1 mEq/L had been co-administered thiazide diuretic and presented with rhabdomyolysis, respectively (Table 5).

DISCUSSION

Occurrence rate of hypokalemia in Kampo-medicines

Both YK preparations contain 1.5 g/day of Glycyrrhiza (Table 1), which is much less than Shakuyakukanzo-to (6.0 g/day of Glycyrrhiza) possessing the highest risk for pseudoaldosteronism among the Kampo-medicines (Table 2).[19-21] However, the Japanese Adverse Drug Event Report (JADER), a spontaneous adverse events reporting system, currently reports that YK-induced pseudoaldosteronism rates are comparable with those of Shakuyakukanzo-to, even though the possible risk should be low in terms of the Glycyrrhiza contents.[19,20] Present results seemed to confirm the JADER's reports; hypokalemia was found in high frequency, with 24.2% of the patients having been treated by YK preparations. This rate is comparable with a previous investigation in elderly patients, where 17% of patients treated with YK developed hypokalemia.[22]

On the other hand, an adverse drug reactions (ADRs) frequency investigation on YK for ethical use reported that hypokalemia occurred in 1.3% patients treated with YK,[13] which was considerably lower than our observation. Several factors may explain this difference in the occurrence rate of hypokalemia. One possible reason is patient background in terms of disease severity, complications and concomitant drug administration. 80% of the subjects in the ADRs investigation were outpatients and 61.9% of the patients had no complications and no medication for dementia.[13] On the other hand, this study enrolled patients who presented with complicating psychiatric disorders (48.8%) and received various medications, including LPIDs such as diuretics, glucocorticoids, mineralocorticoids and

glycyrrhizin preparations. Another possible reason is observation periods between the studies. The ADRs frequency investigation did not track adverse events longer than 52 weeks after starting YK administration.[13]

Hypokalemia in pseudoaldosteronism

Licorice induced-pseudoaldosteronism due to Kampo-medicines can escalate into a serious event that makes hospitalization necessary. The mechanism seems to be clear.[23-26] Glycyrrhetic acid (GA), a metabolite of glycyrrhizin (GL) contained in licorice, has been found to be the major substance for pseudoaldosteronism. GA inhibits 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD 2), which catalyzes the conversion of cortisol to cortisone and prevents the binding of cortisol to the mineralocorticoid receptor (MCR) in the mineralocorticoid target tissues. This inhibition leads to increased cortisol levels in the tissues and excess the cortisol binding to the MCR with same affinity of aldosterone.[23,24] The MCR activation increases sodium reuptake and inhibits potassium reabsorption in the kidney, resulting in hypertension, metabolic alkalosis and hypokalemia.[25] Monitoring of serum potassium levels, therefore, is useful for early detection and assessing the severity of pseudoaldosteronism. Our present results suggested that serum potassium levels should be checked at the first month after starting YK preparations, because the median for hypokalemia onset was 34 days after administration in patients (Table 3). Serum potassium monitoring should be continued during the treatment, because the case of late onset pseudoaldosteronism was found after 3 years administration of Kampo-medicines containing Glycyrrhiza.[27]

Risk factors for YK preparations-induced hypokalemia

We found four risk factors associated with hypokalemia in patients with heterogeneous clinical backgrounds (Table 4). The risk of hypokalemia during YK treatment was 3.09 times

higher than that of YKCH (Table 4). One conceivable explanation of this finding may be due to the difference in GL contents between YK and YKCH even though the Glycyrrhiza contents are the same (1.5 g/day) as shown in Table 1. Kampo-medicines, including YK preparations, were spray-drying products consisting of herbal extracts as showed in Table 1. The mixture of 7 or 9 crude drugs are added to water and boiled, filtered, concentrated and then the resulting decoctions are further spray-dried to yield the dry extract for making YK preparations. In this manufacturing process, the wet extraction rate of GL may be different between YK and YKCH due to difference in the combination of crude drugs and the pH value of their decoction.[28] Higher GL content for YK might therefore present a higher risk of hypokalemia compared with YKCH.

Patients co-administered with LPIDs were 2.74 times more likely to develop to hypokalemia than with YK preparation alone (Table 4, Figure 2). LPIDs co-administration speeds up the hypokalemia onset time compared with YK preparation alone (Figure 2). Among the LPIDs, loop and thiazide diuretics should be carefully noted because they are frequently prescribed for dementia patients with hypertension (data not shown). Severe hypokalemia cases with low potassium levels of 1.9 mEq/L had received thiazide diuretics concomitantly (Table 5).

Patients with hypoalbuminemia had a 2.15 times higher rate of hypokalemia (Table 4). Since 99.9% of circulating GA are bound to albumin,[29] hypoalbuminemia may increase the unbound fraction of GA through pharmacokinetic alteration, resulting in an enhancement of the pharmacological actions of GA. Yoshino T et al. suggested the possibility that hypoalbuminemia was an risk factor for pseudoaldosteronism in three cases receiving other Kampo-medicines. [30] Present results are the first report that hypoalbuminemia is a possible risk factor for licorice-induced hypokalemia in YK preparations.

The occurrence of hypokalemia might be dose dependent in patients treated with YK

preparations, because full-dose YK preparations (7.5 g/day) increased the risk more than 1.60 times compared with a reduced dose (Table 4). This observation is consistent with previous reports suggesting that licorice-induced pseudoaldosteronism was found in a dose-dependent manner.[20,21] Since a majority of the dementia patients taking YK preparations are elderly, the reduced dose is recommended for any patients carrying the risk factors of pseudoaldosteronism. Although age was not identified as a risk factor for hypokalemia in the present study, this might be due a lack of comparison, as most of the patients investigated were elderly (68.6±16.1 years old). Initiation of full-dose YK preparations would therefore be avoided in elderly patients whose 11β-HSD activity might be low due to age-dependent decline in kidney function.[31] In fact, 7 of 9 patients who discontinued YK preparations due to hypokalemia were over 70 years old and carried multiple risk factors of hypokalemia (Table 5).

CONCLUSION

Hypokalemia was found at an unexpectedly high rate in patients under treatment with YK preparations even though the licorice content is relatively small. Four risk factors were found to be important in elderly patients under long term treatment with YK preparations: YK administration, LPIDs co-administration, hypoalbuminemia and full dosage administration (7.5 g/day). It is recommended that serum potassium monitoring should be done at least monthly for safe use of YK preparations in patients with multiple risk factors.

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Contributors:

SS, TA, AT and MH designed and supervised the study. TA and AT selected the patients for this study. SS and MH corrected the data and carried out statistical analyses. SS and MH drafted the original manuscript and all authors checked and revised the manuscript. SS and MH are the guarantors.

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Competing interests: We declare no competing interests.

Ethical approval: This study was approved by The Ethical Committee of the University of Tsukuba Hospital.

Data sharing: The full dataset is available from the corresponding author.

Transparency: The lead author affirms that the manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned have been explained.

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Table 1. Components of YK preparations

Constituent herbs	Weight (g/day)	
	YK [a]	YKCH [b]
JP Atractylodes Lancea Rhizome	4.0	4.0
JP Poria Sclerotium	4.0	4.0
JP Cnidium Rhizome	3.0	3.0
JP Uncaria Hook	3.0	3.0
JP Japanese Angelica Root	3.0	3.0
JP Bupleurum Root	2.0	2.0
JP Glycyrrhiza	1.5	1.5
JP Pinellia Tuber	-	5.0
JP Citrus Unshiu Pee	-	3.0

JP: The Japanese Pharmacopoeia

[a] 7.5g of Tsumura YK extract granules contains 3.25 g of a dried extract of the mixed crude drugs.

[b] 7.5g of Tsumura YKCH extract granules contains 4.5 g of a dried extract of the mixed crude drugs.

Table 2. Commercial available Kampo-medicines containing Glycyrrhiza

Glycyrrhiza contents (g/day)	The number of Kampo-medicines	Examples
6.0	1	Shakuyakukanzo-to
5.0	2	Kanbakutaiso-to, Shakuyakukanzo-bushi-to
3.0	11	Ninjin-to, Oren-to, Shoseiryu-to, etc.
2.5	1	Hangeshashin-to
2.0	38	Kakkon-to, Shosaiko-to, Tokishigyaku-ka-goshuyushokyo-to, etc.
1.5	24	Hochuekki-to, Yokukansan, Yokukansan-ka-chimpihange, etc.
1.0	31	Chotosan, Ninjinyoei-to, Rikkunshi-to, etc.

Table 3. Demographic data of the subjects

	Hypokalemia	Non-hypokalemia	*P* value
Number of patients (male / female)	94 (35/59)	295 (139/156)	0.093
Age (years)	69.5±16.7	68.2±15.9	0.334
Body weight (kg) [a]	51.2±12.8	54.6±14.5	0.182
Disease (dementia / other psychiatric disorder)	42/52	157/138	0.149
YK preparations treatment			
YK / YKCH	86/8 *	237/58	0.012
Full dose	66 (70.2%) *	163 (55.3%)	0.010
Dosing periods (days)	169 (8 to 2280) *	266 (6 to 2788)	0.048
Dosing period until hypokalemia (days)	34 (1-1600)	-	-
Co-administration of LPIDs	36 (38.3%) *	51 (17.3%)	<0.001
Diuretics (loop / thiazide)	10/4	15/7	-
Glucocrticoids / Mineralcorticoid	18/0	23/2	-
Glycyrrhizin preparation	7	18	-
Serum potassium (mEq/L)			
Baseline	4.0±0.3	4.2±0.4	<0.001
Minimum	3.2±0.3 *	4.1±0.3	<0.001
⊿ potassium	-0.7 (-3.0 to -0.1)	-0.1 (-1.3 to 1.1)	<0.001
Laboratory abnormality at baseline [b]			
Aspartate aminotransferase (U/L)	11 (11.7%)	24 (8.1%)	0.293
Alanine aminotransferase (U/L)	14 (14.9%) *	29 (9.8%)	0.009
Albumin (g/dL)	47 (50.0%) *	86 (29.2%)	<0.001
Blood urea nitrogen (mg/dL)	37 (39.4%) *	78 (26.4%)	0.017
Creatinine (mg/dL)	28 (29.8%)	107 (36.3%)	0.250
Sodium (mEq/L)	7 (7.4%)	17 (5.8%)	0.555
Chloride (mEq/L)	11 (11.7%)	39 (13.2%)	0.702

The data are presented as number of patients, mean±S.D. or median (range).
Significant differences were observed : * $p<0.05$ versus non-hypokalemia.
[a] The number of patients whose weight is present is 85 with hypokalemia and 224 with non-hypokalemia.
[b] The number of patients with abnormal laboratory data at baseline.
The normal range for laboratory data are as follows;
Aspartate aminotransferase: 8.0-38.0 U/L,
Alanine aminotransferase: 4.0-44.0 U/L,
Albumin: 3.8-5.3 g/dL,
Blood urea nitrogen: 8.0-20.0 mg/dL,
Creatinine: 0.61-1.04 mg/dL in male and 0.47-0.79 mg/dL in female,
Sodium: 135.0-147.0 mEq/L,
Chloride: 98.0-108.0 mEq/L

Table 4 Hazard ratios for hypokalemia in patients treated with YK preparations*

Risk factors	Univariable analysis		Multivariable analysis	
	Hazard ratio (95% CI)	*P* value	Hazard ratio (95% CI)	*P* value
YK administration (not YKCH)	2.838 (1.373 to 5.866)	0.005	3.093 (1.408 to 6.798)	0.005
LPIDs co-administration	2.877 (1.892 to 4.375)	<0.001	2.743 (1.754 to 4.289)	<0.001
Hypoalbuminemia [a]	2.954 (1.944 to 4.490)	<0.001	2.145 (1.360 to 3.384)	0.001
Full dosed amount	1.636 (1.051 to 2.547)	0.029	1.600 (1.005 to 2.549)	0.048
Female	1.357 (0.893 to 2.061)	0.153	1.316 (0.852 to 2.032)	0.215
Age	1.004 (0.991 to 1.018)	0.537	1.003 (0.989 to 1.017)	0.673
Serum Potassium [a] (≥4.1 mEq/L)	0.358 (0.236 to 0.543)	<0.001	0.450 (0.288 to 0.702)	<0.001

*: Cox proportional hazard model was used. [a]: Determined at baseline.

Table 5. Characteristics of nine patients who were discontinued from YK preparations for hypokalemia

Case	YK preparations	YK preparations dose (g/day)	Dosing period until hypokalemia (days)	Minimum value of serum potassium (mEq/L) (reduction)	Baseline albumin (g/dL)	Symptoms	Co-medication	Number of risk factors
1	YK *	7.5 *	205	1.9 (-2.5)	4.1	Edema	Hydrochlorothiazide *	3
2	YK *	7.5 *	554	2.0 (-3.0)	4.1	Rhabdomyolysis	-	2
3	YK *	7.5 *	24	2.8 (-2.0)	2.5 *	-	-	3
4	YK *	5.0	160	2.8 (-1.4)	-	-	-	1
5	YK *	7.5 *	8	2.9 (-1.7)	2.1 *	-	Rikkunshito [a] *	4
6	YKCH	7.5 *	161	2.9 (-1.1)	3.7 *	-	-	2
7	YK *	5.0	237	3.3 (-0.6)	-	Edema Hypertension	-	1
8	YK *	5.0	26	3.3 (-0.5)	2.6 *	-	-	2
9	YKCH	7.5 *	26	3.5 (-2.5)	3.4 *	-	-	2

* Risk factors for hypokalemia are as follows;
YK, LPIDs, hypoalbuminemia, full dose
[a] Other Kampo-medicine including Glycyrrhiza

FIGURE LEGENDS

Figure 1. The cumulative rate of hypokalemia after administration of YK-preparations.

Figure 2. The effects of LPIDs co-administraition on occurrence of hypokalemia in patients treated with YK-preparations. Solid line: Patients co-administered with LPIDs, Dotted line: Patients co-administered without LPIDs. Significant difference was observed between with and without LPIDs co-administration in the log-rank test ($p<0.001$).

TABLE LEGENDS

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Table 3. Demographic data of the subjects

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Table 5. Characteristics of nine patients who were discontinued from YK preparations for hypokalemia

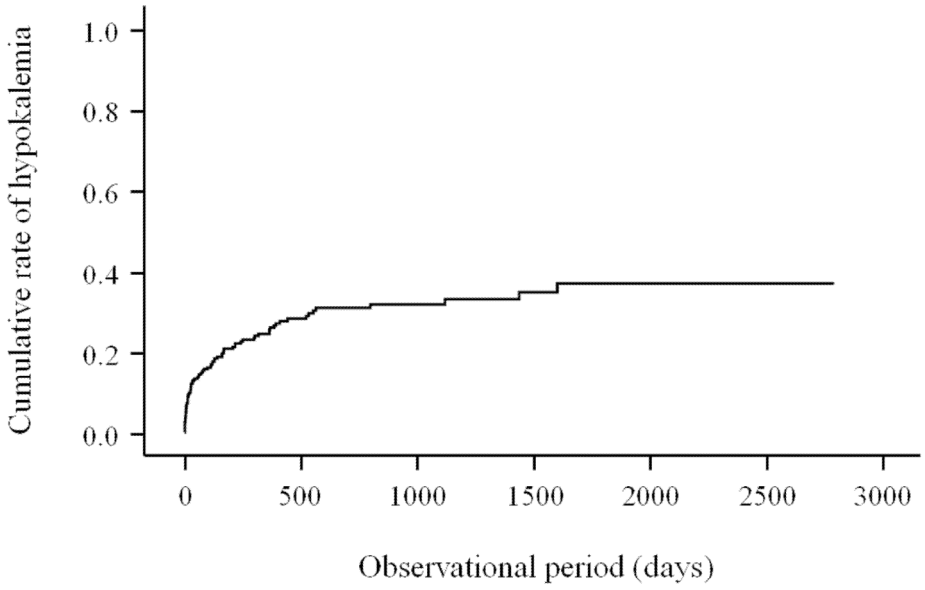

Figure 1. The cumulative rate of hypokalemia after administration of YK-preparations.

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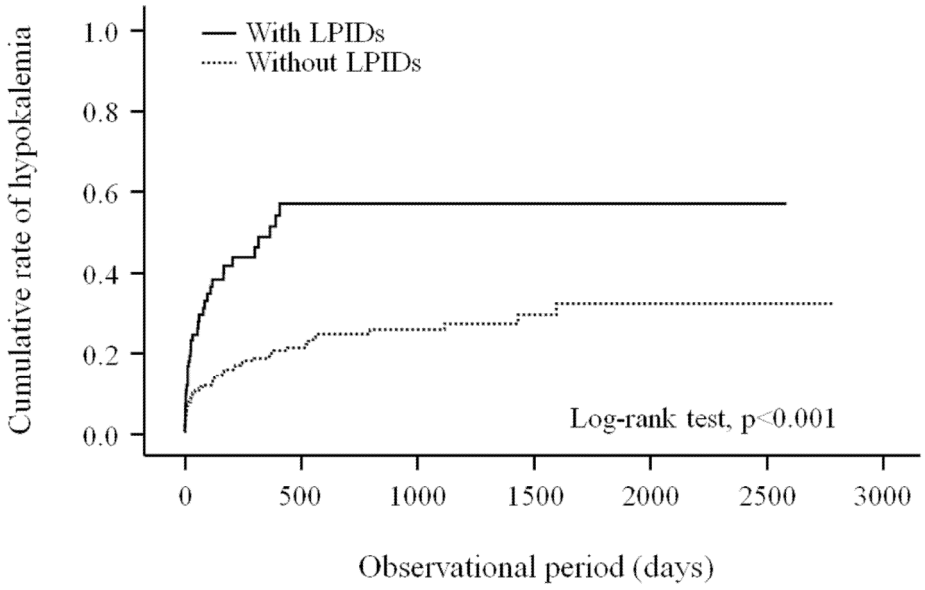

Figure 2. The effects of LPIDs co-administraition on occurrence of hypokalemia in patients treated with YK-preparations. Solid line: Patients co-administered with LPIDs, Dotted line: Patients co-administered without LPIDs. Significant difference was observed between with and without LPIDs co-administration in the log-rank test ($p<0.001$).

420x297mm (300 x 300 DPI)

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation	Reported on page No.
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	Title and Page 2, line 5
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	Page 2, line 1-25
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	Page 4, line 2-21
Objectives	3	State specific objectives, including any prespecified hypotheses	Page 4, line 18-24
Methods			
Study design	4	Present key elements of study design early in the paper	Page 1, line 13-14
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	Page 5, line 12-24
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	Page 5, line 12-16
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	Not applicable
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	Page 5, line 15-19
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	Page 5, line 21-24
Bias	9	Describe any efforts to address potential sources of bias	Not applicable
Study size	10	Explain how the study size was arrived at	Not applicable
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	Page 6, line 5-6
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	Page 6, line 3-9
		(*b*) Describe any methods used to examine subgroups and interactions	Page 6, line 6-8
		(*c*) Explain how missing data were addressed	Not applicable
		(*d*) If applicable, explain how loss to follow-up was addressed	Not applicable
		(*e*) Describe any sensitivity analyses	Not applicable
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	Page 6, line 8-9 and table 3

		(b) Give reasons for non-participation at each stage	Not applicable
		(c) Consider use of a flow diagram	Not applicable
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Page 6, line 16- Page 7 line 10 and table 3
		(b) Indicate number of participants with missing data for each variable of interest	Not applicable
		(c) Summarise follow-up time (eg, average and total amount)	Page 5, line 16-17
Outcome data	15*	Report numbers of outcome events or summary measures over time	Page 6, line 12-15 and figure 1
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Page 7, line 11-18 and table 4
		(*b*) Report category boundaries when continuous variables were categorized	Page 7, line 8-10 and line 16-18
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	Not applicable
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	Page 7, line 19-23
Discussion			
Key results	18	Summarise key results with reference to study objectives	Page 8, line 13-14 and page 9, line 24-25
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	Not applicable
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	Page 8, line 6- page 11, line 12
Generalisability	21	Discuss the generalisability (external validity) of the study results	Page 8, line 6- page 11, line 12
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	Page 12, line 6

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely

available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.

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Licorice-induced hypokalemia in patients treated with Yokukansan preparations—identification of the risk factors in a retrospective cohort study

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Licorice-induced hypokalemia in patients treated with Yokukansan preparations—identification of the risk factors in a retrospective cohort study

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Key words: hypokalemia, pseudoaldosteronism, yokukansan preparations, licorice containing kampo-medicine, risk factors

ABSTRACT

Objective: To evaluate serum potassium and rates of hypokalemia in patients treated with licorice-containing Japanese traditional Kampo-medicines Yokukansan (YK) and Yokukansan-ka-chinpihange (YKCH).

Design: Retrospective cohort study.

Setting: Patients receiving YK preparations for dementia and other psychiatric disorders in the University of Tsukuba Hospital in Japan.

Participants: Three hundred eighty nine patients (male/female: 174/215, 68.6±16.1 years) who were treated with YK preparations for 231 days (range 6 to 2788 days). Patients whose potassium levels were less than 3.6 mEq/L before administration of YK preparations and drug noncompliant patients were excluded.

Main outcome measure: The occurrence rate of hypokalemia and assessment of the risk factor for YK preparation induced hypokalemia.

Results: Out of 389 total patients, 94 (24.2%) developed hypokalemia (potassium levels <3.6 mEq/L) 34 days (range 1 to 1600 days) after administration of YK preparations. Thirty six (38.3%) patients with hypokalemia had co-administration with lower potassium inducing drugs (LPIDs; diuretics, glucocorticoids, mineralcorticoids and glycyrrhizin), which was more frequent in the patients without hypokalemia (17.3%) (p<0.05). A Cox proportional hazard model identified the four risk factors for hypokalemia: YK administration (not YKCH) (hazard ratio 3.093, 95% confidence interval 1.408 to 6.798), co-administration of LPIDs (2.743, 1.754 to 4.289), hypoalbuminemia at baseline (2.145, 1.360 to 3.384) and full dosing amounts (7.5g/day) (1.600, 1.005 to 2.549).

Conclusions: Serum potassium monitoring should be done at least monthly in patients with the risk factors of LPIDs co-administration, YK administration, hypoalbuminemia and full dosage administration.

Strengths and limitations of this study

- This is the first report to identify the risk factors for hypokalemia as an initial symptom of pseudoaldosteronism in patients treated with YK preparations containing small amounts of Glycyrrhiza (1.5g/day).
- Patient data, including backgrounds and laboratory data, are under the sole stewardship of the University of Tsukuba Hospital.
- Since this is a retrospective cohort study, blood sampling intervals for assessing serum potassium and other laboratory data were not fixed.

INTRODUCTION

Yokukansan (YK) preparations YK and Yokukansan-ka-chimpihange (YKCH) are Japanese kampo (traditional) medicines consisting of 7 and 9 crude drug extracts, respectively (Table 1), for the treatment of restlessness and agitation in children.[1] Current use of YK preparations focuses on the treatment of psychological symptoms of dementia (BPSD) in patients with Alzheimer's disease and Lewy body dementia.[1-10] This trend has altered the YK target patient population from children to the elderly in just the past decade.[11] An increase in adverse effects such as liver dysfunction, interstitial pneumonia, pseudoaldosteronism and rhabdomyolysis have been found in dementia patients, leading to the revision of the YK preparation package insert.[12] These adverse effects may be due to the change in target patient age (juvenile to elderly) and interactions with concomitant drugs being administered for the complications.[11,13]

Since both YK preparations contain licorice as the crude drug Glycyrrhiza, they have licorice-induced pseudoaldosteronism characterized by hypertension and hypokalemia as their essential adverse effects.[14] This adverse effect has been ignored to this point because the Glycyrrhiza content of the preparation (1.5 g/day) is less than the 2.5 g/day which is considered to increase the risk of licorice-induced pseudoaldosteronism among Kampo-medicines containing Glycyrrhiza.[15] (Table 2) However, several observations revealed that the occurrence of hypokalemia caused by YK preparations is unexpectedly high and may develop into life-threatening events such as congestive heart failure and rhabdomyolysis, which required cessation of drug administration.[16-18]

In the present study, we retrospectively investigated the change in serum potassium levels in patients treated with YK preparations to assess the risk factor for hypokalemia as an initial symptom of pseudoaldosteronism.

METHODS

YK preparations

YK preparations (YK and YKCH) were obtained in a commercially available granule (Tsumura & Co., Tokyo, Japan) consisting of the extract prepared from the mixture of 7 and 9 crude drugs, respectively (Table1). These traditional medicines are approved for medical use in Japan. The daily dose of both YK preparations is 7.5 g/day as the granule, which contains a dried extract of the mixed crude drugs of YK (3.25 g) or YKCH (4.5 g) as shown in Table 1. Glycyrrhiza content for both YK and YKCH were the same as the daily dose extracts (1.5 g) (Table1, 2).

Patients and Study design

Three hundred eighty-nine patients (174 males and 215 females, mean age 68.6±16.1 years) receiving YK preparations for BPSD of dementia or other psychiatric disorders were enrolled at the University of Tsukuba Hospital from March 2007 to January 2016 (Table 3). 184 patients were treated as outpatients and 205 were admitted during the trial. 323 patients were treated with YK and 66 with YKCH for 231 days (range 6 to 2788 days). YK preparations were given orally before or after meals at full dose strength (2.5 g three times a day; 7.5 g/day) or a reduced dose (2.5 g once or twice a day; 2.5-5.0 g/day) based on patient symptoms. 229 patients (58.9%) received a full dose of YK preparation. Noncompliant patients as well as those whose pre-administration serum potassium level was less than 3.6 mEq/L were excluded from the study. Changes in laboratory data including serum potassium, sodium, chloride, aspartate aminotransferase, alanine aminotransferase, blood urea nitrogen, serum creatinine and albumin and co-medication were retrospectively investigated via medical records before and after administration of YK preparations.

Statistical analyses

Statistical parameters were ascertained by using SPSS software (International Business Machines Corp., Armonk, New York, USA). Statistical analyses were performed by the Mann-Whitney test, and chi-square test for comparing differences between hypokalemic and non-hypokalemic groups. The cutoff threshold for hypokalemia was determined by a Receiver Operating Characteristic curve. Intergroup differences in patients treated with and without lower potassium-inducing drugs (LPIDs; diuretics, glucocorticoids, mineralocorticoids and glycyrrhizin preparations), were analyzed by the Kaplan-Meier method. A Cox proportional hazard model was employed to identify risk factors for hypokalemia. A p value of less than 0.05 was considered to be statistically significant.

RESULTS

94 patients (24.2%) developed hypokalemia (potassium levels: <3.6 mEq/L) during the study period (Table 3). The median time to develop hypokalemia was 34 days (range 1 to 1600 days) after administration of YK preparations (Table 3) at which the cumulative rate of hypokalemia was 12.7% by Kaplan-Meier analysis (Figure 1).

Significant differences between hypokalemic and non-hypokalemic patients were observed and attributed to the type of drug used (YK or YKCH), the dosed amount, and dosing period. Compared to the non-hypokalemic group, the hypokalemic group received YK more often than YKCH (91.5 vs. 80.3%) as well as more full dosing amounts (70.2 vs. 55.3%, p<0.05) (Table 3). The dosing period in hypokalemic cases was significantly shorter than in non-hypokalemic cases (169 days, range 8 to 2280 days vs. 266 days , range 6 to 2788 days, p<0.05) (Table 3).

Of the patients with hypokalemia, 36 (38.3%) were receiving concomitant doses of LPIDs, which was more frequent in the patients without hypokalemia (17.3%) (p<0.05)

(Table 3). Mean serum potassium at baseline in the hypokalemic group was also lower than in non-hypokalemics (p<0.001), even though their values were within the normal range. The reduction for serum potassium (Δ potassium) after administration of YK preparations was remarkable in the hypokalemics compared with the non-hypokalemic group (-0.7: -3.0 to -0.1 vs. -0.1: -1.3 to 1.1, p<0.001) (Table 3).

Hypokalemic patients more often displayed abnormal values in alanine aminotransferase, albumin and blood urea nitrogen at baseline than the non-hypokalemic group (14.9 vs. 9.8%, 50.0 vs. 29.2% and 39.4 vs. 26.4%, p<0.05). A higher rate of hypoalbuminemia (albumin levels: <3.8 g/L) was also observed in hypokalemic patients (45.7 vs. 28.8%, p<0.05) with concomitant minimum potassium levels during the administration of YK preparations (data not shown).

A Cox proportional hazard model based on univariable and multivariable analysis identified four risk factors for YK preparation-induced hypokalemia: YK administration (not YKCH) (hazard ratio 3.093, 95% confidence interval 1.408 to 6.798), co-administration of LPIDs (HR 2.743, 95% CI 1.754 to 4.289), hypoalbuminemia at baseline (HR 2.145, 95% CI 1.360 to 3.384) and full-dose administration of YK preparations (7.5 g/day) (HR 1.600, 95% CI 1.005 to 2.549) (Table 4). On the other hand, baseline potassium levels of ≥ 4.1 mEq/L, established as the optimal threshold to predict non-hypokalemia, were a reverse factor for hypokalemia (HR 0.450, 95% CI 0.288 to 0.702) (Table 4).

To assess the effects of LPID co-administration on the occurrence of hypokalemia, the time between administration of YK preparations and development of hypokalemia was compared between groups with and without LPIDs (Figure 2). Patients treated with concomitant LPIDs showed a shorter time-to-occurrence for hypokalemia than those without concomitant LIPDs (Figure 2) (p<0.001).

Nine patients discontinued YK preparations due to hypokalemia and each possessed

the risk factors indicated in Table 5. Seven patients (except for Case 4 and 7) had multiple risk factors. Cases 1 and 2 developed severe hypokalemia with potassium level < 2.1 mEq/L had been found to have been co-administered a thiazide diuretic or presented with rhabdomyolysis, respectively (Table 5).

DISCUSSION

Occurrence rate of hypokalemia when using YK preparations

Both YK preparations contain 1.5 g/day of Glycyrrhiza (Table 1), which is much less than the Shakuyakukanzo-to (6.0 g/day of Glycyrrhiza) preparation that is thought to possess the highest risk for pseudoaldosteronism among the Kampo-medicines (Table 2).[19-21] However, the Japanese Adverse Drug Event Report (JADER), a spontaneous adverse events reporting system, currently reports that YK-induced pseudoaldosteronism rates are comparable with those of Shakuyakukanzo-to, even though the possible risk should be low in terms of the Glycyrrhiza content.[19,20] Present results seemed to confirm the JADER's reports; hypokalemia was found in high frequency, with 24.2% of the patients having been treated by YK preparations. This rate is comparable with a previous investigation in elderly patients, where 17% of patients treated with YK developed hypokalemia.[22]

On the other hand, an adverse drug reactions (ADRs) frequency investigation on YK for ethical use reported that hypokalemia occurred in 1.3% patients treated with YK,[13] which was considerably lower than our observation. Several factors may explain this difference in the occurrence rate of hypokalemia. One possible reason is patient background in terms of disease severity, complications and concomitant drug administration. 80% of the subjects in the ADRs investigation were outpatients and 61.9% of the patients had no complications and no medication for dementia.[13] On the other hand, this study enrolled patients who presented with complicating psychiatric disorders (48.8%) and received various

medications, including LPIDs such as diuretics, glucocorticoids, mineralocorticoids and glycyrrhizin preparations. Another possible reason is observation periods between the studies. The ADR frequency investigation did not track adverse events longer than 52 weeks after starting YK administration.[13]

Serum potassium monitoring to prevent YK preparations-induced hypokalemia

Licorice induced-pseudoaldosteronism due to Kampo-medicines can escalate into a serious event that makes hospitalization necessary. The mechanism seems to be clear.[23-26] Glycyrrhetic acid (GA), a metabolite of glycyrrhizin (GL) contained in licorice, has been found to be the major substance for pseudoaldosteronism. GA inhibits 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD 2), which catalyzes the conversion of cortisol to cortisone and prevents the binding of cortisol to the mineralocorticoid receptor (MCR) in the mineralocorticoid target tissues. This inhibition leads to increased cortisol levels in the tissues and excess the cortisol binding to the MCR with same affinity of aldosterone.[23,24] The MCR activation increases sodium reuptake and inhibits potassium reabsorption in the kidney, resulting in hypertension, metabolic alkalosis and hypokalemia.[25] Monitoring of serum potassium levels, therefore, is useful for early detection and assessing the severity of pseudoaldosteronism. Our present results suggest that serum potassium levels should be checked the first month after starting YK preparations, because the median time for hypokalemia onset was 34 days after administration (Table 3). Serum potassium monitoring should be continued during treatment, because late onset pseudoaldosteronism was found up to 3 years after final administration of Kampo-medicines containing Glycyrrhiza.[27]

Risk factors for YK preparations-induced hypokalemia

We found four risk factors associated with hypokalemia in patients with heterogeneous clinical backgrounds (Table 4). The risk of hypokalemia during YK treatment was 3.09 times higher than that of YKCH (Table 4). One conceivable explanation of this finding may be due to the difference in GL contents between YK and YKCH even though the Glycyrrhiza content is the same (1.5 g/day) as shown in Table 1. Kampo-medicines, including YK preparations, are spray-dried herbal extracts as shown in Table 1. A mixture of 7 or 9 crude drugs are added to water and boiled, filtered, concentrated and then the resulting decoctions are further spray-dried to yield the extract for making YK preparations. In this manufacturing process, the wet extraction rate of GL may differ between YK and YKCH due to variations in the combination of crude drugs and the pH value of their decoctions.[28] A resultant higher GL content for YK might therefore present a higher risk of hypokalemia compared with YKCH.

Patients co-administered with LPIDs were 2.74 times more likely to develop hypokalemia (Table 4, Figure 2) and experience a shorter time-to-onset compared with YK preparations alone (Figure 2). Among the LPIDs, loop and thiazide diuretics draw special notice because they are frequently prescribed for dementia patients with hypertension (data not shown). Severe hypokalemic cases with low potassium levels of 1.9 mEq/L had received thiazide diuretics concomitantly (Table 5).

Patients with hypoalbuminemia had a 2.15 times higher rate of hypokalemia (Table 4). Since 99.9% of circulating GA are bound to albumin,[29] hypoalbuminemia may increase the unbound fraction of GA through pharmacokinetic alteration, resulting in an enhancement of the pharmacological actions of GA. Yoshino and colleagues have suggested the possibility that hypoalbuminemia was an risk factor for pseudoaldosteronism in three other Kampo-medicine. [30] The present results are the first report that hypoalbuminemia is a possible risk factor for licorice-induced hypokalemia due to YK preparations.

The occurrence of hypokalemia might be dose dependent in patients treated with YK

preparations because full-dose YK preparations (7.5 g/day) increased the risk more than 1.60 times compared with a reduced dose (Table 4). This observation is consistent with previous reports suggesting that licorice-induced pseudoaldosteronism was found in a dose-dependent manner.[20,21] Since a majority of the dementia patients taking YK preparations are elderly, the reduced dose is recommended for any patients carrying the risk factors of pseudoaldosteronism. Although age was not identified as a risk factor for hypokalemia in the present study, this might be due a lack of comparison, as most of the patients investigated were elderly (mean age 68.6±16.1 years old). Initiation of full-dose YK preparations would therefore be avoided in elderly patients whose 11β-HSD activity might be low due to age-dependent decline in kidney function.[31] In fact, 7 of 9 patients who discontinued YK preparations due to hypokalemia were over 70 years old and carried multiple risk factors of hypokalemia (Table 5).

CONCLUSION

Hypokalemia was found at an unexpectedly high rate in patients under treatment with YK preparations even though the licorice content is relatively small. Four risk factors were found to be important in elderly patients under long term treatment with YK preparations: YK versus YKCH administration, LPID co-administration, hypoalbuminemia and full dosage administration (7.5 g/day). It is recommended that serum potassium monitoring should be done at least monthly for safe use of YK preparations in patients with multiple risk factors.

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Contributors:

SS, TA, AT and MH designed and supervised the study. TA and AT selected the patients for this study. SS and MH corrected the data and carried out statistical analyses. SS and MH drafted the original manuscript and all authors checked and revised the manuscript. SS and MH are the guarantors.

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Data sharing: The full dataset is available from the corresponding author.

Transparency: The lead author affirms that the manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned have been explained.

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Table 1. Components of YK preparations

Constituent herbs	Weight (g/day)	
	YK [a]	YKCH [b]
JP Atractylodes Lancea Rhizome	4.0	4.0
JP Poria Sclerotium	4.0	4.0
JP Cnidium Rhizome	3.0	3.0
JP Uncaria Hook	3.0	3.0
JP Japanese Angelica Root	3.0	3.0
JP Bupleurum Root	2.0	2.0
JP Glycyrrhiza	1.5	1.5
JP Pinellia Tuber	-	5.0
JP Citrus Unshiu Peel	-	3.0

JP: The Japanese Pharmacopoeia

[a] 7.5g of Tsumura YK extract granules contains 3.25 g of a dried extract of the mixed crude drugs.

[b] 7.5g of Tsumura YKCH extract granules contains 4.5 g of a dried extract of the mixed crude drugs.

Table 2. Commercial available Kampo-medicines containing Glycyrrhiza

Glycyrrhiza contents (g/day)	The number of Kampo-medicines	Examples
6.0	1	Shakuyakukanzo-to
5.0	2	Kanbakutaiso-to, Shakuyakukanzo-bushi-to
3.0	11	Ninjin-to, Oren-to, Shoseiryu-to, etc.
2.5	1	Hangeshashin-to
2.0	38	Kakkon-to, Shosaiko-to, Tokishigyaku-ka-goshuyushokyo-to, etc.
1.5	24	Hochuekki-to, Yokukansan, Yokukansan-ka-chimpihange, etc.
1.0	31	Chotosan, Ninjinyoei-to, Rikkunshi-to, etc.

Table 3. Demographic data of the subjects

	Hypokalemic	Non-hypokalemic	*P* value
Number of patients (male / female)	94 (35/59)	295 (139/156)	0.093
Age (years)	69.5±16.7	68.2±15.9	0.334
Body weight (kg) [a]	51.2±12.8	54.6±14.5	0.182
Disease (dementia / other psychiatric disorder)	42/52	157/138	0.149
YK preparation treatment			
YK / YKCH	86/8 *	237/58	0.012
Full dose	66 (70.2%) *	163 (55.3%)	0.010
Dosing periods (days)	169 (8 to 2280) *	266 (6 to 2788)	0.048
Dosing period until hypokalemia (days)	34 (1-1600)	-	-
Co-administration of LPIDs	36 (38.3%) *	51 (17.3%)	<0.001
Diuretics (loop / thiazide)	10/4	15/7	-
Glucocrticoids / Mineralcorticoid	18/0	23/2	-
Glycyrrhizin preparation	7	18	-
Serum potassium (mEq/L)			
Baseline	4.0±0.3 *	4.2±0.4	<0.001
Minimum	3.2±0.3 *	4.1±0.3	<0.001
⊿ potassium	-0.7 (-3.0 to -0.1) *	-0.1 (-1.3 to 1.1)	<0.001
Laboratory abnormality at baseline [b]			
Aspartate aminotransferase (U/L)	11 (11.7%)	24 (8.1%)	0.293
Alanine aminotransferase (U/L)	14 (14.9%) *	29 (9.8%)	0.009
Albumin (g/dL)	47 (50.0%) *	86 (29.2%)	<0.001
Blood urea nitrogen (mg/dL)	37 (39.4%) *	78 (26.4%)	0.017
Creatinine (mg/dL)	28 (29.8%)	107 (36.3%)	0.250
Sodium (mEq/L)	7 (7.4%)	17 (5.8%)	0.555
Chloride (mEq/L)	11 (11.7%)	39 (13.2%)	0.702

The data are presented as number of patients, mean±S.D. or median (range).
Significant differences were observed : * $p<0.05$ versus non-hypokalemia.
[a] The number of patients whose weight is present is 85 with hypokalemia and 224 with non-hypokalemia.
[b] The number of patients with abnormal laboratory data at baseline.
The normal range for laboratory data are as follows;
Aspartate aminotransferase: 8.0-38.0 U/L,
Alanine aminotransferase: 4.0-44.0 U/L,
Albumin: 3.8-5.3 g/dL,
Blood urea nitrogen: 8.0-20.0 mg/dL,
Creatinine: 0.61-1.04 mg/dL in male and 0.47-0.79 mg/dL in female,
Sodium: 135.0-147.0 mEq/L,
Chloride: 98.0-108.0 mEq/L

Table 4 Hazard ratios for hypokalemia in patients treated with YK preparations*

Risk factors	Univariable analysis		Multivariable analysis	
	Hazard ratio (95% CI)	*P* value	Hazard ratio (95% CI)	*P* value
YK administration (not YKCH)	2.84 (1.37 to 5.87)	0.005	3.09 (1.41 to 6.80)	0.005
LPIDs co-administration	2.88 (1.89 to 4.38)	<0.001	2.74 (1.75 to 4.29)	<0.001
Hypoalbuminemia [a]	2.95 (1.94 to 4.49)	<0.001	2.15 (1.36 to 3.38)	0.001
Fully-dosed amount	1.64 (1.05 to 2.55)	0.029	1.60 (1.01 to 2.55)	0.048
Female	1.36 (0.89 to 2.06)	0.153	1.32 (0.85 to 2.03)	0.215
Age	1.00 (0.99 to 1.02)	0.537	1.00 (0.99 to 1.02)	0.673
Serum Potassium [a] (≥4.1 mEq/L)	0.36 (0.24 to 0.54)	<0.001	0.45 (0.29 to 0.70)	<0.001

*: Cox proportional hazard model was used. [a]: Determined at baseline.

Table 5. Characteristics of nine patients who were discontinued from YK preparations due to hypokalemia

Case	YK preparations	YK preparations dose (g/day)	Dosing period until hypokalemia (days)	Minimum value of serum potassium (mEq/L) (reduction)	Baseline albumin (g/dL)	Symptoms	Co-medication	Number of risk factors
1	YK *	7.5 *	205	1.9 (-2.5)	4.1	Edema	Hydrochlorothiazide *	3
2	YK *	7.5 *	554	2.0 (-3.0)	4.1	Rhabdomyolysis	-	2
3	YK *	7.5 *	24	2.8 (-2.0)	2.5 *	-	-	3
4	YK *	5.0	160	2.8 (-1.4)	-	-	-	1
5	YK *	7.5 *	8	2.9 (-1.7)	2.1 *	-	Rikkunshito [a] *	4
6	YKCH	7.5 *	161	2.9 (-1.1)	3.7 *	-	-	2
7	YK *	5.0	237	3.3 (-0.6)	-	Edema Hypertension	-	1
8	YK *	5.0	26	3.3 (-0.5)	2.6 *	-	-	2
9	YKCH	7.5 *	26	3.5 (-2.5)	3.4 *	-	-	2

* Risk factors for hypokalemia are as follows;
YK, LPIDs, hypoalbuminemia, full dose
[a] Other Kampo-medicine including Glycyrrhiza

FIGURE LEGENDS

Figure 1. The cumulative rate of hypokalemia after administration of YK preparations.

Figure 2. The effects of LPID co-administration on occurrence of hypokalemia in patients treated with YK preparations. Solid line: Patients co-administered with LPIDs, Dotted line: Patients without LPID co-administration. A significant difference was observed between patients with and without LPID co-administration in the log-rank test ($p<0.001$).

TABLE LEGENDS

Table 1. Components of YK preparations

Table 2. Commercial available Kampo-medicines containing Glycyrrhiza

Table 3. Demographic data of the subjects

Table 4 Hazard ratios for hypokalemia in patients treated with YK preparations*

Table 5. Characteristics of nine patients who were discontinued from YK preparations for hypokalemia

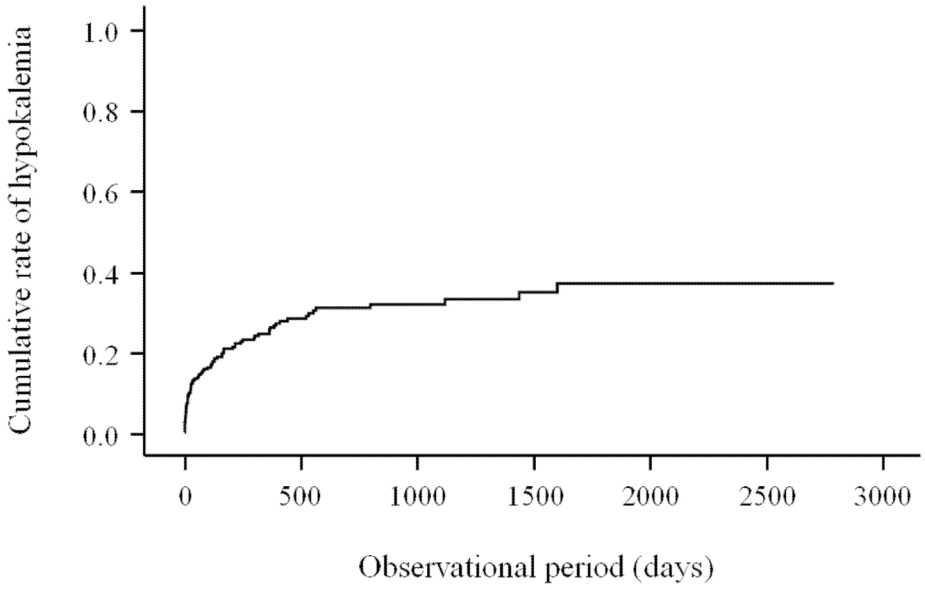

Figure 1. The cumulative rate of hypokalemia after administration of YK-preparations.

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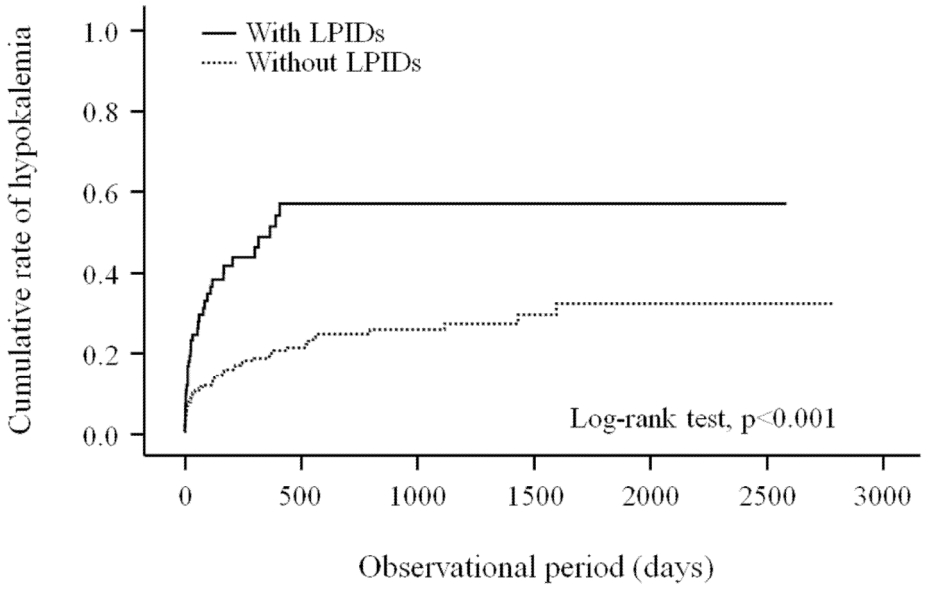

Figure 2. The effects of LPIDs co-administraition on occurrence of hypokalemia in patients treated with YK-preparations. Solid line: Patients co-administered with LPIDs, Dotted line: Patients co-administered without LPIDs. Significant difference was observed between with and without LPIDs co-administration in the log-rank test ($p<0.001$).

420x297mm (300 x 300 DPI)

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation	Reported on page No.
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	Title and Page 2, line 5
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	Page 2, line 1-25
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	Page 4, line 2-21
Objectives	3	State specific objectives, including any prespecified hypotheses	Page 4, line 18-24
Methods			
Study design	4	Present key elements of study design early in the paper	Page 5, line 12-14
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	Page 5, line 12-24
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	Page 5, line 12-16
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	Not applicable
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	Page 5, line 15-19
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	Page 5, line 21-24
Bias	9	Describe any efforts to address potential sources of bias	Not applicable
Study size	10	Explain how the study size was arrived at	Not applicable
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	Page 6, line 5-6
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	Page 6, line 3-10
		(*b*) Describe any methods used to examine subgroups and interactions	Page 6, line 6-8
		(*c*) Explain how missing data were addressed	Not applicable
		(*d*) If applicable, explain how loss to follow-up was addressed	Not applicable
		(*e*) Describe any sensitivity analyses	Not applicable
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	Page 6, line 13-14 and table 3

		(b) Give reasons for non-participation at each stage	Not applicable
		(c) Consider use of a flow diagram	Not applicable
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Page 6, line 17- Page 7 line 11 and table 3
		(b) Indicate number of participants with missing data for each variable of interest	Not applicable
		(c) Summarise follow-up time (eg, average and total amount)	Page 5, line 15-16 and page 6, line 21-23
Outcome data	15*	Report numbers of outcome events or summary measures over time	Page 6, line 13-16 and figure 1
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Page 7, line 12-19 and table 4
		(*b*) Report category boundaries when continuous variables were categorized	Page 7, line 8-11 and line 17-19
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	Not applicable
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	Page 7, line 20-24
Discussion			
Key results	18	Summarise key results with reference to study objectives	Page 8, line 14-17 and page 10, line 1-2
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	Not applicable
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	Page 8, line 7- page 11, line 12
Generalisability	21	Discuss the generalisability (external validity) of the study results	Page 8, line 7- page 11, line 12
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	Page 12, line 8

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.